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ENDOGENOUS PROSTAGLANDINS AND STIMULATED GASTRIC SECRETION IN THE CAT: THE EFFECT OF VARIOUS SECRETORY INHIBITORS

By R. BAKER, B. M. JAFFE, the late J. D. REED, B. SHAW and C. W. VENABLES

From the Departments of Surgery and Physiology, University of Newcastle upon Tyne and Washington University, St Louis, Mo., U.S.A.

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SUMMARY

1. The effects of cimetidine, somatostatin and atropine upon the outputs of acid, pepsin, prostaglandins (PG) E and F in gastric juice secreted in response to I.V. infusions of pentagastrin and histamine (H) have been studied in the conscious gastric fistula cat.

2. At constant rate infusions secretion of PGE occurs and follows a similar pattern to that of gastric acid. However the ratio of H:PGE varies considerably and to an extent inexplicable in terms of assay variation.

3. Inhibition of acid output is matched by inhibition of the output of PGE, but changes in concentration of PGE suggest this is a volume related phenomenon.

4. It is concluded that gastric juice PGE is most unlikely to have a regulatory effect upon acid secretion.

INTRODUCTION

PGE is synthesized by the gastric mucosa and secreted into gastric juice (Cocearni, Pace-Asciak, Volta & Wolfe, 1967; Pace-Asciak & Wolfe, 1970; Pace-Asciak, 1972), but its physiological role remains uncertain. PGE is a potent inhibitor of gastric secretion (Nezamis, Robert & Stowe, 1971; Carter, Karim, Bhana & Ganesan, 1973) and it has been suggested that PGE may be involved in the local control of gastric acid secretion (Ramwell & Shaw, 1968; Horton, 1969). Recently we have demonstrated similar positive correlations between acid and PGE in gastric juice secreted in response to pentagastrin, histamine and insulin (Baker, Jaffe, Reed, Shaw & Venables, 1978). To further evaluate the relationships between stimulated gastric acid and PGE outputs the effects of the inhibitors cimetidine, somatostatin and atropine have been studied.

METHODS

Experimental design

Experiments were performed in the same six conscious cats (mean wt. 3·05 kg) with cannulated gastric fistulae prepared at least 1 year earlier. The animals were fasted for 36 hr before each experiment but allowed free access to water. A percutaneous needle (Butterfly 21G, Abbott Ltd) was inserted into a cephalic vein and 0·9 % saline infused at 12 ml.hr^{-1}. After two 15 min basal periods, gastric secretion was stimulated on separate occasions by 3 hr infusions of pentagastrin (Peptavlon, I.C.I., England), 8 μg kg^{-1} hr^{-1} and histamine acid phosphate (B.D.H.,

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England), 250 μg kg^{-1} hr^{-1}. To determine the effects of the inhibitors cimetidine and somatostatin, these substances were infused, at rates of 2 mg kg^{-1} hr^{-1} and 5 μg kg^{-1} hr^{-1} respectively, for the second hour only of the infusions of pentagastrin and histamine acid phosphate. The effect of atropine upon pentagastrin stimulated gastric secretion was studied by infusing atropine 100 μg kg^{-1} hr^{-1} for the second hour only immediately following the bolus injection of atropine 100 μg kg^{-1}. Gastric secretion was collected continuously and measured at 15 min intervals. Each experiment acted as its own control, however the secretory response to pentagastrin and histamine alone have been previously described in this journal (Baker *et al.* 1978).

Biochemical analysis

Gastric acid output was determined by electrometric titration of 1 ml. samples of gastric juice to pH 7·0 with 0·1 N-NaOH (Radiometer, Denmark) and expressed as μequiv kg^{-1} 15 min^{-1}. Pepsin-like activity was measured by a haemoglobin digestion method (Chiang, Sanchez-Chiang. Wolf & Tang, 1966) and calculated as μg equivalent to bovine pepsin kg^{-1} 15 min^{-1}. Following storage at − 20 °C, 1 ml. samples of gastric juice were extracted by the addition of 3·0 ml. of an organic solvent solution (ethyl acetate : isopropanol : 0·1 N-HCl, 3 : 3 : 1) and two phases created by the addition of 2·0 ml. ethyl acetate and 3·0 ml. water. The organic phases were aspirated, evaporated at 37 °C and dissolved in benzene : ethyl acetate : methanol, 60 : 40 : 2. Fractions containing prostaglandins of the E and F series were eluted separately from microsilicic acid columns by increasing concentrations of methanol (2 % for PGEs and 17 % for PGFs respectively) (Jaffe & Parker, 1972). Concentrations of immunoreactive prostaglandins E and F were measured using specific homologous radioimmunoassay systems (Jaffe, Behrman & Parker, 1973). Antibodies to PGE were raised in rabbits immunized with PGE_1 conjugated to Keyhole Limpet haemocyanin using ethyl chloroformate (Jaffe, Smith, Newton & Parker, 1971); antibodies to PGF were elicited in rabbits immunized with $PGF_{2\alpha}$ conjugated to bovine serum albumin using a water-soluble carbodiimide (Jaffe *et al.* 1971). Tritiated markers ([^{3}H]PGE_1 and [^{3}H]$PGF_{2\alpha}$) were purchased from the New England Nuclear Company and stored at − 20 °C under nitrogen until utilization. Separation of antibody bound and free labelled ligand was performed using dextran-coated charcoal.

Each of the antibodies demonstrates specificity for the cyclopentane ring and the combined immunoassay-chromatographic separation of PGEs and PGFs from each other, and from PGAs, PGBs and thromboxane B_2, is > 99 %. However the assay does not distinguish between PGE_1, and PGE_2, and $PGF_{2\alpha}$ and $PGF_{1\alpha}$. The sensitivity of each radioimmunoassay is 5 pg ml.$^{-1}$. Intra- and interassay coefficients of variation are less than 10 %.

Analysis of data

Gastric acid, pepsin and prostaglandin were expressed in terms of output (μequiv, μg and pg kg^{-1} 15 min^{-1} respectively) and concentration (μequiv, μg and pg ml.$^{-1}$ respectively), as the mean ± 1 S.E. of mean. Significance of differences between corresponding means was calculated by Student's t test for paired data. Values of P less than 0·05 were considered significant.

RESULTS

Pentagastrin stimulation

Peak acid output was attained at 30–45 min following the commencement of the pentagastrin infusion and was not significantly different on any occasion, the peak acid outputs being 485 ± 53, 502 ± 75 and 484 ± 60 μequiv kg^{-1} 15 min^{-1} respectively ($\bar{x}$ ± 1 S.E. of mean) (Figs. 1, 3, 5). Peak pepsin output corresponded to peak acid output, the respective values of 1725 ± 544, 2136 ± 813 and 1036 ± 255 μg kg^{-1} 15 min^{-1} being not significantly different. The peak outputs of PGE also corresponded to peak acid output, the outputs being 786 ± 101, 1007 ± 154 and 361 ± 97 pg kg^{-1} 15 min^{-1} respectively, but the latter peak output was significantly lower than the others.

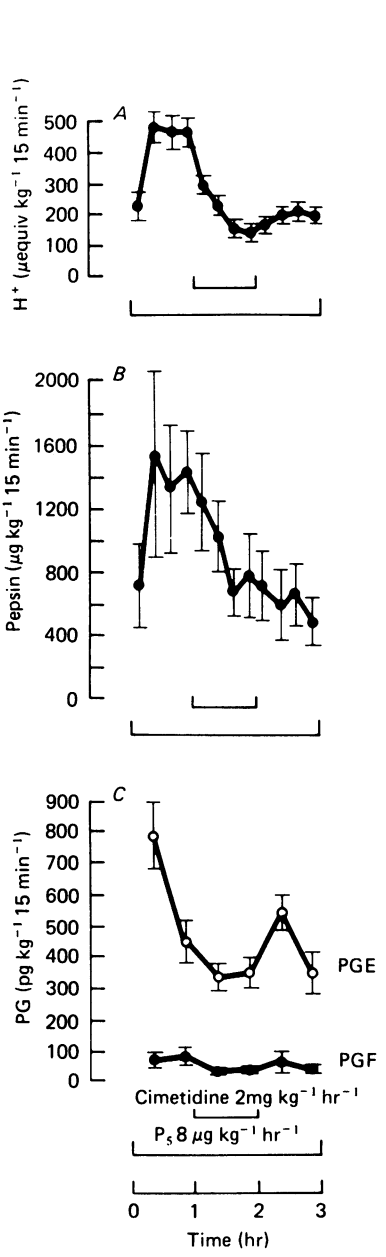

Fig. 1

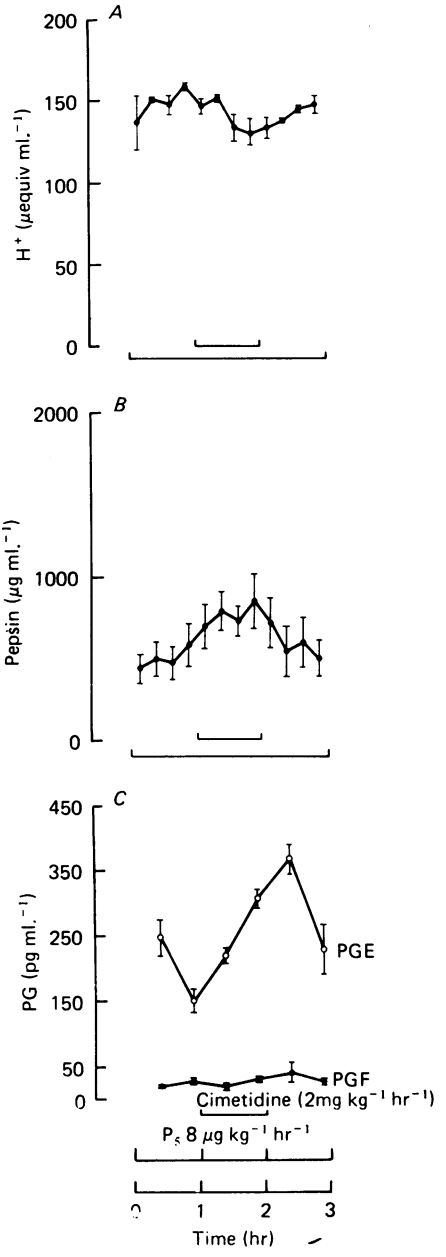

Fig. 2

Fig. 1. The gastric outputs of acid (*A*), pepsin (*B*) and prostaglandins (PG) E and F (*C*) during a 3 hr infusion of pentagastrin (P_5 8 $\mu g\ kg^{-1}\ hr^{-1}$) with the simultaneous infusion of cimetidine (2 mg $kg^{-1}\ hr^{-1}$) during the second hour only. Responses in this Figure and all subsequent Figures are means ± 1 S.E. of mean of observations during one experiment performed in the same six conscious cats.

Fig. 2. The concentrations of acid (*A*), pepsin (*B*) and prostaglandins (PG) E and F (*C*) during a 3 hr infusion of pentagastrin (P_5 8 $\mu g\ kg^{-1}\ hr^{-1}$) with the simultaneous infusion of cimetidine (2 mg $kg^{-1}\ hr^{-1}$) during the second hour only. The secretory outputs are shown in Fig. 1.

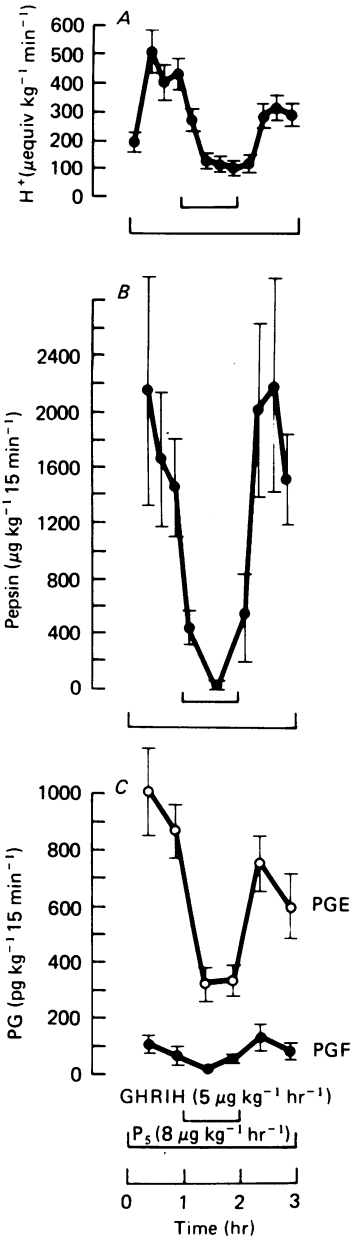

Fig. 3

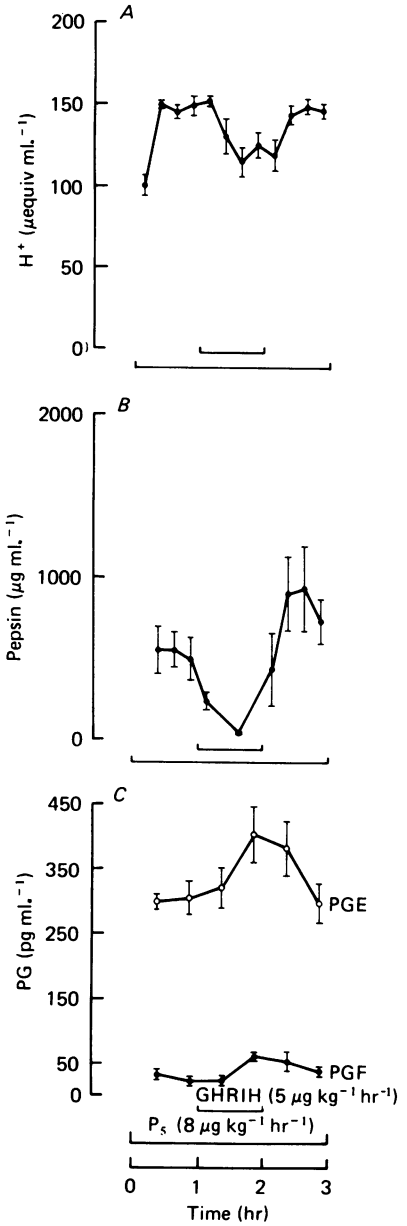

Fig. 4

Fig. 3. The gastric outputs of acid (*A*), pepsin (*B*) and PGE and PGF (*C*) during a 3 hr infusion of pentagastrin (P_5 8 $\mu g\ kg^{-1}\ hr^{-1}$) with the simultaneous infusion of somatostatin (GHRIH 5 $\mu g\ kg^{-1}\ hr^{-1}$) during the second hour only. Six cats.

Fig. 4. The concentrations of acid (*A*), pepsin (*B*) and PGE and PGF (*C*) during a 3 hr infusion of pentagastrin (P_5 8 $\mu g\ kg^{-1}\ hr^{-1}$) with the simultaneous infusion of somatostatin (GHRIH 5 $\mu g\ kg^{-1}\ hr^{-1}$) during the second hour only. The secretory outputs are shown in Fig. 3.

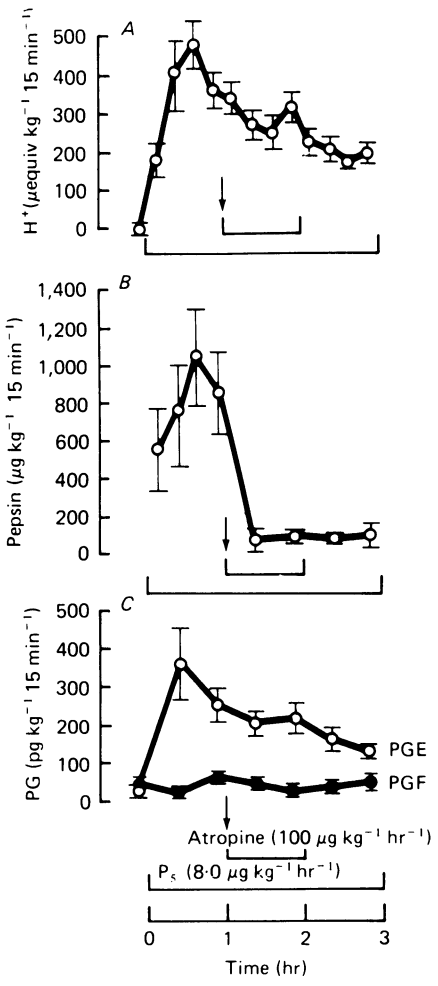

Fig. 5

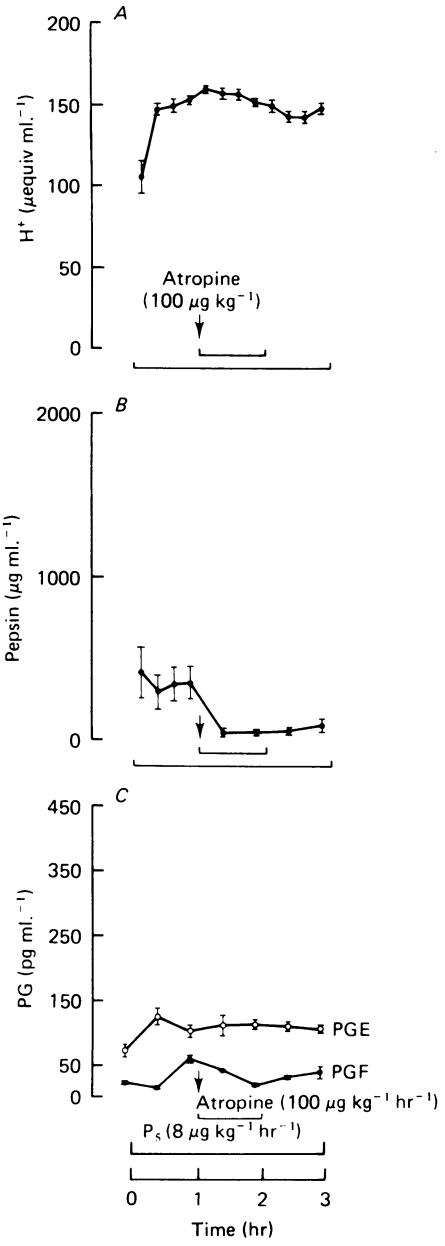

Fig. 6

Fig. 5. The gastric outputs of acid (*A*), pepsin (*B*) and PGE and PGF (*C*) during a 3 hr infusion of pentagastrin (P_5 8 $\mu g\ kg^{-1}\ hr^{-1}$) with the simultaneous infusion of atropine (100 $\mu g\ kg^{-1}\ hr^{-1}$) during the second hour only and immediately following the bolus injection of atropine (100 $\mu g\ kg^{-1}$) (↓). Six cats.

Fig. 6. The concentrations of acid (*A*), pepsin (*B*) and PGE and PGF (*C*) during a 3 hr infusion of pentagastrin (P_5 8 $\mu g\ kg^{-1}\ hr^{-1}$) with the simultaneous infusion of atropine (100 $\mu g\ kg^{-1}\ hr^{-1}$) during the second hour only and immediately following the bolus injection of atropine (100 $\mu g\ kg^{-1}$) (↓). The secretory outputs are shown in Fig. 5.

Cimetidine 2 mg kg^{-1} hr^{-1} inhibited the output of both acid and PGE, but not that of pepsin (Fig. 1). However PGE concentration remained either unaltered or elevated (Fig. 2). Somatostatin 5 μg kg^{-1} hr^{-1} produced profound inhibition of the outputs of acid, pepsin and PGE (Fig. 3), but once again PGE concentration was either unaltered or elevated (Fig. 4). Atropine 100 μg kg^{-1} I.V. followed by 100 μg kg^{-1} hr^{-1} had no significant effect on the outputs of acid or PGE, but pepsin output fell markedly (Fig. 5). PGE concentration following atropine remained unaltered (Fig. 6).

Histamine stimulation

In both experiments peak acid output occurred within the first hour of the histamine infusion and these were not significantly different (422 ± 75 and 426 ± 84 μequiv kg^{-1} 15 min^{-1} respectively) (Figs. 7, 9). Peak pepsin levels were also similar in both experiments (209 ± 125 and 389 ± 203 μg kg^{-1} 15 min^{-1}) but as expected were much lower than during pentagastrin stimulation, as histamine is known to be a poor stimulant of pepsin secretion in the cat. During the first hour of the histamine infusion similar peak levels of PGE occurred (577 ± 75 and 684 ± 124 pg kg^{-1} 15 min^{-1} respectively).

Cimetidine 2 mg kg^{-1} hr^{-1} inhibited acid output by approximately 50% while not significantly affecting pepsin output (Fig. 7). There was a transient inhibition of PGE output which was followed by the progressive rise of PGE previously described during histamine stimulation (Baker *et al.* 1978) (Fig. 8). During the period of inhibition concentrations of PGE were either unaltered or raised (Fig. 8). Somatostatin 5 μg kg^{-1} hr^{-1} produced no inhibition of gastric acid secretion but pepsin secretion was virtually abolished (Fig. 9). No inhibition of PGE output occurred, the output of PGE continuing to rise before some reduction towards the end of the experiment (Figs. 9, 10).

DISCUSSION

We have previously described similar significant positive correlations between acid and PGE in gastric juice secreted in response to pentagastrin, histamine and insulin (Baker *et al.* 1978). However, the possibility exists that these correlations were spurious volume related phenomena. In the present study the patterns of outputs of PGE and acid are again seen to be similar; but inhibition of acid secretion, which is seen to occur as a marked fall in secretory volume with a slight fall in H^+ concentration, is generally accompanied by elevation of PGE concentration. It would appear that the previously described correlations between acid and PGE are indeed spurious. This is further supported by wide variations in the H^+:PGE ratio which occur throughout the experiments and especially during the periods when the secretagogues only are being unfused.

These results suggest that intraluminal PGE has no regulatory effect upon acid secretion. Furthermore if one assumes that gastric juice prostaglandin levels reflect mucosal prostaglandin metabolism then no support is provided for the proposed negative feed-back mechanism involving PGE in the control of acid secretion.

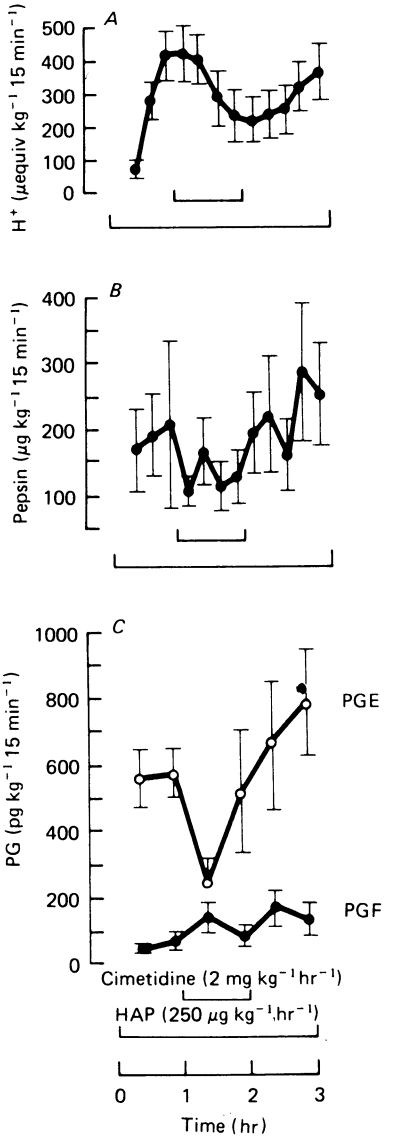

Fig. 7

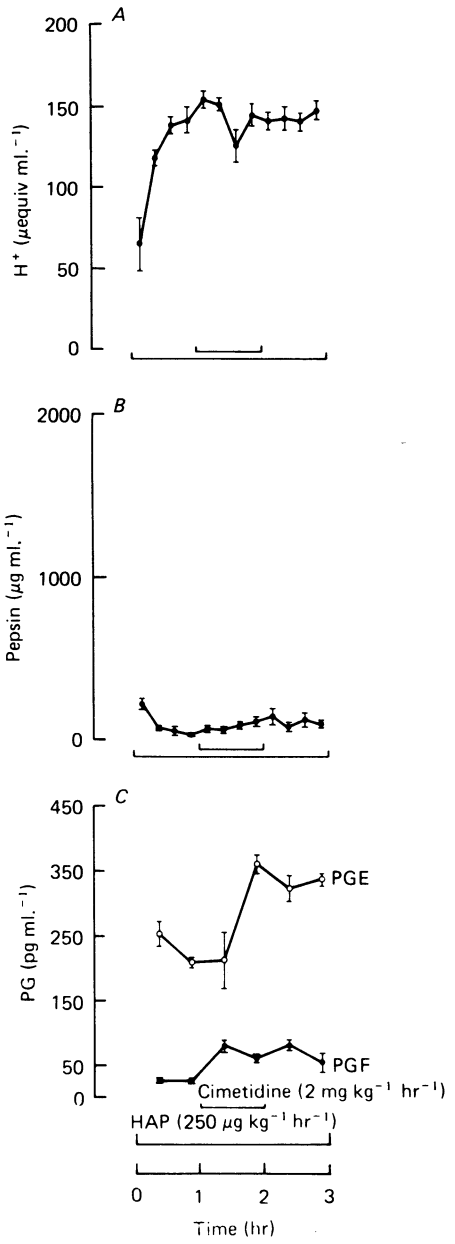

Fig. 8

Fig. 7. The gastric outputs of acid (*A*), pepsin (*B*) and PGE and PGF (*C*) during a 3 hr infusion of histamine acid phosphate (HAP 250 μg kg^{-1} hr^{-1}) with the simultaneous infusion of cimetidine (2 mg kg^{-1} hr^{-1}) during the second hour only. Six cats.

Fig. 8. The concentrations of acid (*A*), pepsin (*B*) and PGE and PGF (*C*) during a 3 hr infusion of histamine acid phosphate (HAP 250 μg kg^{-1} hr^{-1}) with the simultaneous infusion of cimetidine (2 mg kg^{-1} hr^{-1}) during the second hour only. The secretory outputs are shown in Fig. 7.

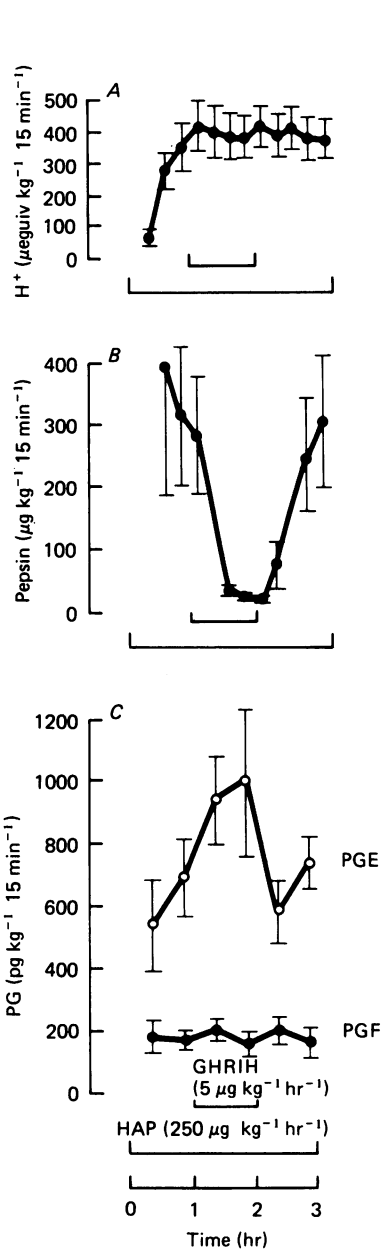

Fig. 9

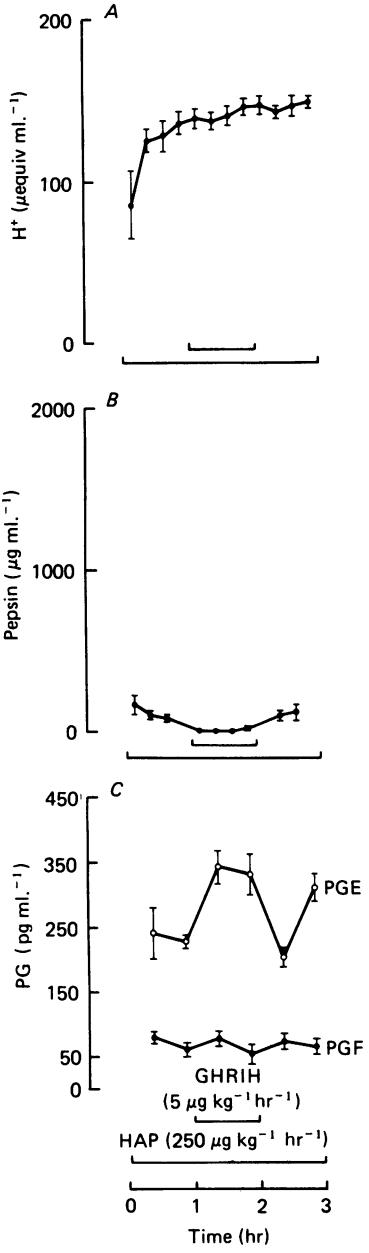

Fig. 10

Fig. 9. The gastric outputs of acid (*A*), pepsin (*B*) and PGE and PGF (*C*) during a 3 hr infusion of histamine acid phosphate (HAP 250 μg kg^{-1} hr^{-1}) with the simultaneous infusion of somatostatin (GHRIH 5 μg kg^{-1} hr^{-1}) during the second hour only. Six cats.

Fig. 10. The concentrations of acid (*A*), pepsin (*B*) and PGE and PGF (*C*) during a 3 hr infusion of histamine acid phosphate (HAP 250 μg kg^{-1} hr^{-1}) with the simultaneous infusion of somatostatin (GHRIH 5 μg kg^{-1} hr^{-1}) during the second hour only. The secretory outputs are shown in Fig. 9.

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